Modestobacter italicus sp. nov., isolated from Carrara marble quarry and emended descriptions of the genus *Modestobacter* and the species *Modestobacter marinus, Modestobacter multiseptatus, Modestobacter roseus and Modestobacter versicolor*

Maria del Carmen Montero-Calasanz[1*], Adnan Yaramis[1], Imen Nouioui[1], José Mariano Igual[2], Cathrin Spröer[3], Jean Franco Castro[4], Peter Schumann[3], Hans-Peter Klenk[1], Clara Urzì[5]

[1]School of Natural and Environmental Sciences, Newcastle University, Ridley Building 2, Newcastle upon Tyne, NE1 7RU, United Kingdom.

[2]Instituto de Recursos Naturales y Agrobiología de Salamanca, Consejo Superior de Investigaciones Científicas (IRNASA-CSIC), c/Cordel de Merinas 40-52, 37008 Salamanca, Spain

[3]Leibniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures, Inhoffenstraße 7B, 38124 Braunschweig, Germany.

[4]Centre for Biotechnology and Bioengineering (CeBiB), Department of Chemical Engineering and Biotechnology, University of Chile, Beauchef 851, Santiago, Chile.

[5]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Italy.

*To whom correspondence should be addressed. E-mail: maria.montero-calasanz@ncl.ac.uk,

Phone: +44 (0) 191 208 4943

Running title: *Modestobacter italicus*, sp. nov. and emended descriptions.

The journal's contents category (New taxa-Actinobacteria)

The GenBank/EMBL/DDBJ whole-genome sequence accession number of strain BC 501^T=DSM 44449^T=CECT 9708^T is FO203431. The GenBank/EMBL/DDBJ 16S rRNA gene sequence accession number is MK020151.

A Gram reaction-positive, aerobic bacterial strain showing coccoid cells and designated as BC 501^T was isolated from a black patina of Carrara marble block surface in the Gioia quarry in Tuscany, Italy. A polyphasic study was carried out to clarify the taxonomic status of strain BC 501^T within the evolutionary radiation of the genus *Modestobacter*. Phenotypic and genotypic characteristics as well as phylogenetic distinctiveness confirmed that it represents a novel species in the genus *Modestobacter*, for which the name *Modestobacter italicus* sp. nov. is proposed. The type strain is BC 501^T (= DSM 44449^T= CECT 9708^T). Emended descriptions of the genus *Modestobacter* and the species *Modestobacter marinus*, *Modestobacter multiseptatus*, *Modestobacter roseus* and *Modestobacter versicolor* are also proposed.

Keywords: *Geodermatophilaceae*, digital DDH, phenotyping, oxidative stress

The genus *Modestobacter* [1], of the family *Geodermatophilaceae* [2], encompasses seven validly named species: *Modestobacter multiseptatus* [1], as the type species, *Modestobacter caceresii* [3], *Modestobacter lapidis* [4], *Modestobacter marinus* [5], *Modestobacter muralis* [4], *Modestobacter roseus* [6], and *Modestobacter versicolor* [7] and an effectively published species '*Modestobacter lacusdianchii*' [8]. Representatives of the genus are recognised by a combination of chemotaxonomic, morphological and physiological properties [3] and can be distinguished from other genera in *Geodermatophilaceae* by 16S rRNA phylogenetic inference [8] and their polar lipids profiles [9]. Representatives of *Geodermatophilaceae* are well known for their tolerance to oxidative stress and hence well adapted to harsh environmental conditions [3, 10, 11, 12, 13]. Species in *Modestobacter* were mainly isolated from oligotrophic ecosystems such as Antarctica [1], desert soils [3], deep sea sediments [5] or deteriorated sandstones [4], although some were also recovered from others environments such as biocrusts [7], plants roots [6] and algae mats [8].

Monuments and rocks surfaces exposed both indoor and outdoor are peculiar and poorly studied habitats inhabited by a wide range of Gram-positive strains belonging to the class *Actinobacteria* [14] frequently classified as representing novel taxa [15, 16, 17, 18]. Strain BC 501^T was isolated from a Carrara marble block at Gioia quarry in Tuscany, Italy [19]. Urzì *et al*. [19] already showed

that it clustered into the genus *Modestobacter*. In particular, strain BC 501^T showed distinctive characteristics compared to the only, at that time, described species *M. multiseptatus* and hence the authors proposed that it should be considered as a new species. Later on Gtari *et al*. [12] and Sghaier *et al*. [13] revealed the differential adaptation to stress associated with arid environment in *Geodermatophilaceae* and its correlation to colonization patterns observed, and misnamed the strain BC 501^T as *M. multiseptatus.* Normand *et al*. [20] renamed this strain as *M. marinus* on the basis of the high percentage of similarity (99.5 %) between the 16S rRNA gene sequences of strain BC 501^T and the *M. marinus* type strain described by Xiao *et al*. [5]. However, several phenotypic and genotypic differences lead us to still confirm that strain BC 501^T is a new species within the genus *Modestobacter*. This paper therefore aims, through a polyphasic approach, the taxonomic characterisation of strain BC 501^T as the type strain of a novel species in the genus *Modestobacter*, to be named *M. italicus*. In addition, based on new chemotaxomic data obtained during the course of this work, we propose the emendation of the genus *Modestobacter* and species *M. marinus*, *M. multiseptatus, M. roseus* and *M. versicolor.*

Cultural features of strain BC 501^T were tested on GYM *Streptomyces* medium (DSMZ medium 65), PYGV medium (DSMZ medium 621), and Luedemann's medium (DSMZ medium 877). Morphological characteristics were examined in GYM *Streptomyces* broth (DSMZ medium 65), shaken (180 r.p.m.) at 28 °C for 4 days by optical microscopy (Zeiss AxioScope A1) with a 100-fold magnification and phase-contrast illumination. Gram reaction was carried out by the KOH test described by Gregersen [21]. Phenotypic profiles of strain BC 501^T and the reference strains *M. caceresii* KNN 45-2b^T, '*M. lacusdianchii'* KCTC 39600, *M. lapidis* MON 3.1^T, *M. marinus* DSM 45201^T, *M. multiseptatus* DSM 44406^T, *M. muralis* MDVD1^T, *M. roseus* DSM 45764^T and *M. versicolor* DSM 16678^T grown on GYM *Streptomyces* agar plates at 28 °C for 4 days were comparatively analysed in duplicate using GEN III Microplates in an Omnilog device (BIOLOG Inc., Hayward, CA, USA) at 28 °C. Cell suspensions were prepared in a viscous inoculating fluid (IF C) provided by the manufacturer at 83 % transmittance (T) for all strains except for '*M. lacusdianchii'* that was at 95 % T. Data were exported and analysed using the opm package for R v.1.0.6 [22, 23]. Reactions with a distinct behaviour between the two replicates were regarded as ambiguous. Additional phenotypic data including temperature, pH and salt ranges were already determined by Urzì *et al.* [19]. Those were added here to the protologue for completion of the characterisation of the novel species.

For all chemotaxonomic tests except fatty acids analysis, strains were cultivated in GYM *Streptomyces* broth, shaken (180 r.p.m.) at 28 °C for 7 days, and, consequently, cell material was collected and freeze-dried. Whole-cell amino acids and sugars were prepared according to

Lechevalier & Lechevalier [24], followed by thin-layer chromatography (TLC) analysis [25]. The composition of peptidoglycan hydrolysates (6 N HCl, 100 ºC for 16 h) was analysed by TLC on cellulose plates as described by Schleifer & Kandler [26]. Menaquinones (MK) were extracted using the procedures of Collins *et al.* [27] and analysed by high-performance liquid chromatography (HPLC) [28]. Polar lipids were extracted and then identified by two-dimensional TLC as described by Minnikin *et al.* [29] with modifications proposed by Kroppenstedt & Goodfellow [30]. Moreover, choline-containing lipids were detected by spraying with Dragendorff's reagent (Merck Millipore, 102035) [31]. For analysis of cellular fatty acids, strains were grown on PYGV (Peptone-Yeast extract-Glucose-Vitamins; DSM medium 621) agar plates for 16 days at 20 °C and biomass from the last quadrant streak was harvested. The extraction and identification of fatty acids methyl esters were obtained following the protocol described by Sasser [32] and using the Microbial Identification System (MIDI) Sherlock Version 6.1 (method TSBA40, ACTIN6 database). The annotation of the fatty acids in the ACTIN6 peak naming table is consistent with IUPAC nomenclature (*i.e.* double bond positions identified with reference to the carboxyl group of the fatty acid), but for consistency with other publications this has been altered to numbering from the aliphatic end of the molecule (*i.e.* $C_{16:1}$ CIS 9 becomes $C_{16:1}$ ω7c and $C_{17:1}$ CIS 9 becomes $C_{17:1}\omega$8c).

Genomic DNA extraction, PCR-mediated amplification of the 16S rRNA gene, and purification of the PCR product were carried out as described by Rainey *et al.* [33]. The taxonomic affiliation of strain BC 501^T was determined based on its 16S rRNA sequence using EZBioCloud (http://www.ezbiocloud.net/taxonomy)[34]. Phylogenetic analyses were conducted under maximum-likelihood (ML) and maximum-parsimony (MP) as optimality criteria using RAxML version 7.2.8 [35] and PAUP* 4b10 [36] using the bootstopping criterion [37] as implemented in RAxML and 1000 replicates in the case of PAUP* as previously described [38] using the DSMZ phylogenomics pipeline [39] adapted to single genes integrated in the GGDC web server [40] available at http://ggdc.dsmz.de/. The rooting of the inferred tree was determined by the midpoint method [41]. Pairwise similarities were calculated as recommended by Meier-Kolthoff *et al.* [42] for the 16S rRNA gene available via the GGDC web server. For DNA-DNA hybridization tests, cells of strains BC 501^T and *Modestobacter marinus* DSM 44509^T was disrupted by using a Constant Systems TS 0.75 KW (IUL Instruments, Germany). DNA in the crude lysate was purified by chromatography on hydroxyapatite as described by Cashion *et al.* [43]. Wet lab DNA-DNA hybridization was carried out as described by De Ley *et al.* [44] under consideration of the modifications described by Huss *et al.* [45] using a model Cary 100 Bio UV/VIS-spectrophotometer equipped with a Peltier-thermostatted 6x6 multicell changer and a temperature controller with *in*

situ temperature probe (Varian). The digital DNA–DNA hybridization (dDDH) values between the genome of strain BC 501^T [20] and genomes of *M. caceresii* DSM 101691^T, *'M. lacusdianchii'* KCTC 39600 (Montero-Calasanz *et al*., unpublished data), *M. muralis* DSM 100205^T (Sangal and Goodfellow, unpublished data), *M. roseus* DSM 45764^T and *M. versicolor* DSM 16678^T (Sangal and Goodfellow, unpublished data) were calculated using the genome-to-genome distance calculator, GGDC 2.0 [40, 46].

Strain BC 501^T was a Gram-reaction-positive actinobacterium. Young colonies were light red-coloured and turned to black at maturity. Colonies were convex, nearly circular and opaque with a moist surface and an entire margin. Cell morphology was characterized by non-motile cocci of 1-1.5 µm diameter tending to form aggregates as described for the genus *Modestobacter* [1]. Bud formations were occasionally observed. Strain BC 501^T grew well on GYM *Streptomyces*, PYGV and Luedemann's media. As indicated by Urzì *et al.* [19], it tolerated temperature ranging from 26 to 37 °C with an optimum at 30 °C. Growth occurred at 1 % NaCl, but not at 3-8 % NaCl, and between pH 5.0-10.0 with an optimal pH range of 8.5. A summary of selected differential phenotypic characteristics is presented in Table 1. Full GEN III phenotype is shown as a heatmap in Supplementary Fig. 1.

Whole-cell hydrolysates of strain BC 501^T contained *meso*-diaminopimelic acid (cell wall type III) [24], which is consistent with the affiliation to the family *Geodermatophilaceae* [46]. Whole-cell sugar analysis of strain BC 501^T revealed ribose, arabinose, mannose, glucose, and galactose as diagnostic sugar. A similar pattern was also observed for *M. roseus* DSM 45764^T, although in the original species description by Qin *et al.* [6] just the presence of ribose, glucose, and galactose was noted. Similarly, the whole-cell extracts of *M. versicolor* DSM 16678^T revealed the presence of ribose, glucose, galactose, rhamnose, and traces of mannose. The presence of rhamnose was already described by Busarakam *et al.* [3] for *M. caceresii*. The absence of galactose was observed in the sugars profile of *M. marinus* DSM 45201^T, and *M. multiseptatus* DSM 44406^T, contrarily to the whole-cell sugars pattern outlined by Mevs *et al*. [1]. In addition, traces of ribose and mannose were also identified for *M. marinus* DSM 45201^T and *M. multiseptatus* DSM 44406^T, respectively.

Predominant menaquinone (> 50 %) was MK-9(H_4) (73.7 %) in accordance with data reported for the family *Geodermatophilaceae* [2, 9, 47], but MK-8(H_4) (7.3 %) and MK-9(H_2) (1.9 %) were also detected. MK-8(H_4) (9.9 %) was besides observed in *M. roseus* DSM 45764^T but not in *M. multiseptatus* DSM 44406^T, contrarily to the data shown by Mevs *et al*. [1]. MK-9(H_2) (11.5 %) was identified in *M. versicolor* DSM 16678^T. The presence of MK-9 was otherwise detected in significant amounts in *M. versicolor* DSM 16678^T (21.2 %), *M. roseus* DSM 45764^T (5.4 %), and

M. marinus DSM 45201^T (4.7 %). Menaquinone MK-10(H_4) was, in addition, a minor component (1.4 %) in the menaquinones profile of *M. marinus* DSM 45201^T. The presence of MK-9(H_6) (35.8 %) already described by Mevs *et al*. [1] for *M. multiseptatus* DSM 44406^T was also confirmed in this study. Major cellular fatty acids (>5 %) of strain BC 501^T were iso-$C_{16:0}$ (32.8 %) and $C_{18:1}\omega9c$ (17.4 %) in agreement with the major fatty acids observed for other species in the genus under standardised conditions (See Supplementary Table S1 for full profiles). Diphosphatidylglycerol, phosphatidylinositol, glycophosphatidylinositol and phosphatidylethanolamine were the major polar lipids of strain BC 501^T with their chromatographic mobility documented in Figure 1a. In addition, an unidentified aminolipid and three unidentified lipids were also detected. This is in accordance with the chromatographic profiles observed in this study for the references strains (Fig1b-e) and what was described by Qin *et al.* [6] for the genus *Modestobacter.* Distinctive taxonomic features can be nevertheless observed between species. The presence of glycophosphatidylinositol (annotated as phosphatidylinositol mannoside (PIM) by Qin *et al.* [6], Trujillo *et al.* [4] and Busarakam *et al.* [3]) is a common characteristic observed in the chromatographic profiles of all the species, except for *M. multiseptatus* DSM 44406^T, in which it switches to an unidentified glycolipid. Similar change was already observed in other species belonging to the family *Geodermatophilaceae* [9, 47]. In addition, it is worth mentioning the chromatographic pattern formed by phosphatidylinositol, an unidentified phospholipid and glycophosphatidylinositol (annotated as phosphatidylinositol mannoside by other authors) previously revealed by Trujillo *et al.* [4] and Busarakam *et al.* [3] for the species *M. muralis*, *M. lapidis* and *M. caceresii* is common to *M. marinus* DSM 45201^T and *M. versicolor* DSM 16678^T.

Based on the complete (1508 bp) 16S rRNA gene sequence (GenBank/EMBL/DDBJ accession number MK020151) and both Maximum Likelihood and Maximum Parsimony estimations, strain BC 501^T was placed within a phylogenetic group containing type strains of all effectively published species of the genus *Modestobacter* with very high support (Fig. 2). The 16S rRNA gene sequence showed the highest similarity with the homologous genes of *M. caceresii* KNN 45-2b^T (99.8 %), *M. marinus* 42H12-1^T (99.6 %), *M. roseus* KLBMP 1279^T (99.6 %), *M. versicolor* CP153-2^T (99.5 %), *M. muralis* MDVD1^T (99.3 %), *'M. lacusdianchii'* JXJ CY 19 (99.3 %), *M. lapidis* MON 3.1^T (98.7 %), and *M. multiseptatus* AA-826^T (98.6 %). Meier-Kolthoff *et al.* [41] tied Actinobacteria-specific 16S rRNA threshold of 99.0 % with 1.0 % as maximum probability of error, to DNA–DNA hybridization (DDH) values above the 70 % threshold required to assign a given strain to a new species [48]. Based on that argument, *in silico* DDH values with the type strains of *M. caceresii*, *M. marinus*, *M. roseus*, *M. versicolor*, *M. muralis,* and the proposed type strain of *'M. lacusdianchii'*

were determined and corresponded to 26.4 %, 40.6 ± 4.2 % (wet DDH), 25.7 %, 30.6 %, 26.3 %, and 27.5 %, respectively. The G+C content of the DNA of strain BC 501^T was 74.1 % (Normand *et al*., 2012).

The 16S rRNA gene sequence and digital DNA–DNA relatedness together with some physiological and chemotaxonomic differences (Table 1) clearly warrant the proposal of a novel species to accommodate strain BC 501^T, for which the name *Modestobacter italicus* sp. nov. is proposed.

Based on a review of the literature and new data obtained in this study, emended descriptions of the genus *Modestobacter* and the species *M. marinus, M. multiseptatus, M. roseus* and *M. versicolor* were also provided.

Emended description of the genus *Modestobacter* Mevs *et al.* 2000 emend. Reddy *et al.* 2007 emend. Xiao *et al*. 2011 emend. Qin *et al*. 2013

The properties are as given by Mevs *et al.* (2000) and emended by Reddy *et al.* (2007), Xiao *et al*. (2011), and Qin *et al*. (2013) with the following emendations regarding chemotaxonomic properties. The diagnostic diamino acid is, as previously described, *meso*-diaminopimelic acid. The basic polar lipids profile involves diphosphatidylglycerol, phosphatidylethanolamine, and phosphatidylinositol. The presence of unidentified compounds like a glycophospholipid (alternatively a glycolipid), phospholipids, aminophospholipids, lipids, and aminolipids is frequent. The characteristic whole-cell sugar pattern consists of ribose and glucose. The presence of arabinose, mannose, rhamnose, and galactose is variable. The predominant menaquinone is usually MK-9(H_4) but MK-9(H_6) could appear as the primary menaquinone in some species. MK-8(H_4), MK-9, MK-9(H_2), and MK-10(H_4) may also be present.

The type species is *Modestobacter multiseptatus*.

Emended description of *Modestobacter marinus* Xiao *et al*. 2011

The properties are as given in the species description by Xiao *et al*. 2011 with the following emendation. In addition to diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, phosphatidylglycerol, and the unidentified aminophospholipid listed by Xiao *et al*. 2011, the polar lipids profile contains an unidentified glycophospholipid and an unidentified phospholipid (the chromatographic mobility of which are documented in Fig. 1b). The whole-cell sugars consist of arabinose, mannose, glucose, and traces of ribose. The predominant menaquinone is MK-9(H_4) but MK-9 and MK-10(H_4) are present as minor components.

The type strain is 42H12-1^T = DSM 45201^T = CGMCC 4.5581^T

Emended description of *Modestobacter multiseptatus* Mevs *et al.* (2000) emend. Reddy *et al.* 2007

The properties are as given in the species description by Mevs *et al.* (2000) and emended by Reddy *et al*. (2007) with the following emendation. In addition to diphosphatidylglycerol, phosphatidylethanolamine, and phosphatidylinositol listed by Reddy *et al*. 2007, an unidentified glycolipid, two unidentified aminolipids, and three unidentified lipids are present (the chromatographic mobility of which is documented in Fig. 1c). Phosphatidylglycerol listed by Reddy *et al*. (2007) is absent. In addition to glucose and ribose listed by Mevs *et al.* (2000), the whole-cell sugars consist of traces of mannose. The presence of galactose, as listed by Mevs *et al.* (2000), was not detected. MK-9(H_4) and MK-9(H_6) are the predominant menaquinones, MK-8(H_4) listed by Mevs *et al.* (2000) is absent.

The type strain is AA-826^T = CIP 106529^T = DSM 44406^T = JCM 12207^T

Emended description of *Modestobacter roseus* Qin *et al.* 2013

The properties are as given in the species description by Qin *et al.* (2013) with the following emendation. In addition to diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol and, phosphatidylinositol mannoside (annotated here as glycophosphatidylinositol) listed by Qin *et al.* (2013), the polar lipids profile contains phosphatidylglycerol, an unidentified aminolipid, and two unidentified aminophospholipids (the chromatographic mobility of which is documented in Fig. 1d). The two unidentified aminophospholipids listed by Qin *et al.* (2013) were absent**.** In addition to ribose, glucose, and galactose listed by Qin *et al.* (2013), the whole-cell sugars consist of arabinose and mannose. MK-9(H_4) is the predominant menaquinone but MK-9 and MK-8(H_4) are present as minor components. The genomic G+C content is 74.5 %. The genome size is 4.5 Mbp.

The IMG accession number for the whole genome sequence of the type strain DSM 45764^T is 2585427561.

The type strain is KLBMP 1279^T = KCTC 19887^T = NBRC 108673^T = DSM 45764^T

Emended description of *Modestobacter versicolor* Reddy *et al*. 2007

The properties are as given in the species description by Reddy *et al*. (2007) with the following emendation. In addition to diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, and phosphatidylinositol listed by Reddy *et al*. (2007) and Xiao *et al*. (2011), the polar lipids profile contains an unidentified phospholipid, glycophosphoinositol, and four unidentified lipids (the chromatographic mobility of which are documented in Fig.1e). The whole-cell sugars consist of rhamnose, ribose, glucose, galactose, and traces of mannose. MK-9(H_4) is the predominant menaquinone, but MK-9 and MK-9(H_2) are present as minor components.

The type strain is CP153-2^T = ATCC BAA-1040^T = DSM 16678^T

Description of *Modestobacter italicus* sp. nov.

Modestobacter italicus (i.ta′li.cus, L. masc. adj. *italicus* from Italy, where the bacterium was first isolated).

Colonies are black-coloured, opaque with a moist surface and regular margin. Cells are Gram-reaction-positive. Negative for gelatine and casein degradation. Nitrate is reduced to nitrite. Positive for starch degradation. Temperature for growth is 26-37 °C and pH 5.0-10.0. NaCl is not needed for growth; the strain can grow in the presence of 1 % of NaCl but not up to 3 % of NaCl. According to the Biolog System, it oxidises dextrin, D-cellobiose, turanose, D-glucose, D-mannose, L-rhamnose, sodium lactate, D-mannitol, glycerol, rifamycin SV, minocycline, L-galactonic acid-γ-lactone, nalidixic acid, potassium tellurite, tween 40, aztreonam, butyric acid and sodium bromate but not D-maltose, β-gentiobiose, D-raffinose, α-D-lactose, D-melibiose, β-methyl-D-glucoside, *N*-acetyl-D-glucosamine, *N*-acetyl-β-D-mannosamine, *N*-acetyl-D-galactosamine, *N*-acetyl-neuraminic acid, 3-*O*-methyl-D-glucose, D-fucose, L-fucose, inosine, fusidic acid, D-sorbitol, D-arabitol, myo-inositol, D-glucose-6-phosphate, D-aspartic acid, D-serine, troleandomycin, gelatin, glycine-proline, L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, L-histidine, L-pyroglutamic acid, L-serine, lincomycin, guanidine hydrochloride, niaproof, pectin, D-galacturonic acid, D-glucuronic acid, glucuronamide, mucic acid, D-saccharic acid, vancomycin, tetrazolium violet, tetrazolium blue, p-hydroxy-phenylacetic acid, methyl pyruvate, D-lactic acid methyl ester, L-lactic acid, citric acid, α-keto-glutaric acid, D-malic acid, L-malic acid, bromo-succinic acid, lithium chloride, γ-amino-n-butyric acid, α-hydroxy-butyric acid, β-hydroxy-butyric acid, α-keto-butyric acid,

propionic acid, acetic acid and sodium formate. The major menaquinone (> 50 %) is MK-9(H_4) but MK-8(H_4) and MK-9(H_2) are also found. Dominant fatty acids (> 10 %) are iso-$C_{16:0}$ and $C_{18:1}\omega 9c$. The peptidoglycan in the cell wall contains *meso*-diaminopimelic acid. Ribose, arabinose, mannose, glucose and galactose are the whole-cell sugars. Polar lipids consist of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol and glycophosphatidylinositol. Genomic DNA G + C content 74.1 %.

The INSDC accession number for the whole-genome sequence of the type strain BC 501^T (= DSM 44449^T= CECT 9708^T) is FO203431. The GenBank/EMBL/DDBJ 16S rRNA gene sequence accession number is MK020151.

Funding Information

IN is grateful for a postdoctoral fellowship from Newcastle University and JFC for support from the Newton Project for UK–Chile collaboration (Grant JIC CA586) and for the Basal Programme of CONICYT for funding of the Centre for Biotechnology and Bioengineering, CeBiB (project FB0001). MdCM-C was the recipient of a DSMZ postdoctoral fellowship 2013–2015.

Acknowledgments

We would like to gratefully acknowledge the help of Bettina Sträubler and Birgit Grün (DSMZ, Braunschweig) with DNA-DNA hybridization analysis and Vartul Sangal and Michael Goodfellow by providing us the *in silico* DNA-DNA hybridization values between our strain and *M. versicolor* and *M. muralis*. We also thank Dr. Martha E. Trujillo (University of Salamanca, Spain) by providing us the references strains *M. lapidis* MON 3.1^T and *M. muralis* MDVD1^T. '*M. lacusdianchii*' KCTC 39600 was supplied by KCTC. Other reference strains were supplied by DSMZ GmbH.

Conflict of Interest

The authors declare that there are no conflicts of interest.

References

1. **Mevs U, Stackebrandt E, Schumann P, Gallikowski CA, Hirsch P.** *Modestobacter multiseptatus* gen. nov., sp. nov., a budding actinomycete from soils of the Asgard Range (Transantarctic Mountains). *Int J Syst Evol Microbiol* 2000;50: 337-346.

2. **Normand P.** *Geodermatophilaceae* fam. nov., a formal description. *Int J Syst Evol Microbiol* 2006;56: 2277-2278.
3. **Busarakam K, Bull AT, Trujillo ME, Riesco R, Sangal V, *et al.*** *Modestobacter caceresii* sp. nov., novel actinobacteria with an insight into their adaptive mechanisms for survival in extreme hyper-arid Atacama Desert soils. *Syst App Microbiol* 2016;39: 243-251.
4. **Trujillo M, Goodfellow M, Busarakam K, Riesco R.** *Modestobacter lapidis* sp. nov. and *Modestobacter muralis* sp. nov., isolated from a deteriorated sandstone historic building in Salamanca, Spain. *Antonie van Leeuwenhoek* 2015;108: 311-320.
5. **Xiao J, Luo Y, Yu J, Xie J.** *Modestobacter marinus* sp. nov., a psychrotolerant actinobacterium from deep-sea, and emended description of the genus *Modestobacter*. *Int J Syst Evol Microbiol* 2011;61:1704-17010.
6. **Qin S, Bian G-K, Zhang Y-J, Ying K, Cao C-L, *et al.*** *Modestobacter roseus* sp. nov., an endophytic actinomycete isolated from the coastal halophyte *Salicornia europea* Linn, and emended description of the genus *Modestobacter*. *Int J Syst Microbiol* 2013;63: 2197-2202.
7. **Reddy GSN, Potrafka RM, Garcia-Pichel F.** *Modestobacter versicolor* sp. nov., an actinobacterium from biological soil crusts that produces melanins under oligotrophy, with emended descriptions of the genus *Modestobacter* and *Modestobacter multiseptatus* Mevs et al. 2000. *Int J Syst Evol Microbiol* 2007;57:2000-2014.
8. **Zhang B-H, Salam N, Cheng J, Li H-Q, Yang J-Y, *et al.*** *Modestobacter lacusdianchii* sp. nov., a phosphate-solubilizing actinobacterium with ability to promote *Microcystis* growth. PLoS ONE 11(8): e0161069. doi:10.1371/ journal.pone.0161069
9. **Montero-Calasanz MdC, Meier-Kolthoff JP, Zhang D-F, Yaramis A, Rohde M, *et al*.** Genome-Scale Data Call for a Taxonomic Rearrangement of *Geodermatophilaceae*. *Front Microbiol* 2017; 8;2501. https://doi.org/10.3389/fmicb.2017.02501
10. **Montero-Calasanz MdC, Göker M, Broughton WJ, Cattaneo A, Favet J, *et al*.** *Geodermatophilus tzadiensis* sp. nov., a UV radiation-resistant bacterium isolated from sand of the Saharan desert. *Syst Appl Microbiol 2013;* 36, 177-182. doi: 10.1016/j.syapm.2012.12.005
11. **Montero-Calasanz MdC, Hofner B, Göker M, Rohde M, Spröer C, *et al.*** *Geodermatophilus poikilotrophi* sp. nov., a multi-tolerant actinomycete isolated from dolomitic marble. *Biomed Res Int* 2014; 914767. doi: 10.1155/2014/914767
12. **Gtari M, Essoussi I, Maaoui R, Sghaier H, Boujmil R, et al.** Contrasted resistance of stone dwelling *Geodermatophilaceae* species to stresses known to give rise to reactive oxygen species. *FEMS Microbiol Ecol* 2012;80: 566-577.

13. **Sghaier H, Hezbri K, Gohdhbane-Gtari F, Pujic P, Sen A, *et al*.** Stone-dwelling actinobacteria *Blastococcus saxobsidens*, *Modestobacter marinus* and *Geodermatophilus obscurus* proteogenomes. *The ISME Journal* 2016;10: 21-29.
14. **Sterflingerc K, Piñar G.** Microbial deterioration of cultural heritage and works of art — tilting at windmills? *Appl Microbiol Biotechnol*.2013; 97: 9637-9646. doi: 10.1007/s00253-013-5283-1
15. **Urzì C, Salamone P, Schumann P, Rohde M, Stackebrandt E**. *Blastococcus saxobsidens* sp. nov., and emended descriptions of the genus *Blastococcus* Ahrens and Moll 1970 and *Blastococcus aggregatus* Ahrens and Moll 1970. *Int J Syst Evol Microbiol* 2004;54: 253-259.
16. **Alias-Villegas C, Jurado V, Laiz L, Miller AZ, Saiz-Jimenez C.** *Nocardioides albertanoniae* sp. nov., isolated from Roman catacombs. *Int J Syst Evol Microbiol* 2013;63:1280-1284.
17. **Everest GJ, Curtis SM, De Leo F, Urzì C, Meyers PR.** Description of *Kribbella italica* sp. nov., isolated from a roman catacomb. *Int J Syst Evol Microbiol* 2015;65: 491-496.
18. **Groth I, Schumann P, Schuetze B, Augsten K, Kramer I, *et al.*** *Beutenbergia cavernae* gen. nov., sp. nov., an L-lysine-containing actinomycete isolated from a cave. *Int J Syst Bacteriol* 2009;49: 1733-1740.
19. **Urzì C, Brusetti L, Salamone P, Sorlini C, Stackebrandt E, *et al.*** Biodiversity of *Geodermatophilaceae* isolated from altered stones and monuments in the Mediterranean basin. *Environ Microbiol* 2001;3:471-479.
20. **Normand P, Gury J, Pujic P, Chouaia B, Crott E, *et al.*** Genome sequence of radiation-resistant *Modestobacter marinus* strain BC 501, a representative actinobacterium that thrives on calcareous stone surfaces. *J Bacteriol* 2012;194: 4773-4774.
21. **Gregersen T.** Rapid method for distinction of gram-negative from positive bacteria. *Appl Microbiol Biotechnol* 1978;5: 123-127.
22. **Vaas LAI, Sikorski J, Hofer B, Fiebig A, Buddruhs N *et al*.** opm: An R package for analysing OmniLog Phenotype Microarray data. *Bioinformatics* 2013;29:1823-1824.
23. **Vaas LAI, Sikorski J, Michael V, Göker M, Klenk H-P.** Visualization and curve-parameter estimation strategies for efficient exploration of phenotype microarray kinetics. *PLoS ONE* 2012;7, e34846.
24. **Lechevalier MP, Lechevalier H.** Chemical composition as a criterion in the classification of aerobic actinomycetes. *Int J Syst Bacteriol* 1970;20: 435-443.
25. **Staneck JL, Roberts GD.** Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. *Appl Microbiol* 1974;28: 226-231.

26. **Schleifer KH, Kandler.** Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol Rev* 1972;36: 407-477.
27. **Collins MD, Pirouz T, Goodfellow M, Minnikin DE.** Distribution of menaquinones in actinomycetes and corynebacteria. *J Gen Microbiol* 1977;100: 221-230.
28. **Kroppenstedt RM.** Separation of Bacterial Menaquinones by HPLC Using Reverse Phase (RP18) and a Silver Loaded Ion Exchanger as Stationary Phases. *J Liq Chromatogr* 1982;5: 2359-2367. Taylor & Francis Group.
29. **Minnikin DE, O'Donnell AG, Goodfellow M, Alderson G, Athalye M *et al*.** An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* 1984;2: 233-241.
30. **Kroppenstedt RM, Goodfellow M (2006).** The family *Thermomonosporaceae*: *Actinocorallia, Actinomadura, Spirillispora* and *Thermomonospora*. In: Dworkin M, Falkow S, Schleifer KH, Stackebrandt E (editors). *The prokaryotes*, 3rd edn. New York: Springer; 2006. pp. 682-724.
31. **Tindall BJ.** A comparative study of the lipid composition of *Halobacterium saccharovorum* from various sources. *Syst Appl Microbiol* 1990;13: 128-130.
32. **Sasser M.** Identification of Bacteria by Gas Chromatography of Cellular Fatty Acids. MIDI Technical Note *101*. Newark, DE: MIDI Inc; 1990.
33. **Rainey FA, Ward-Rainey N, Kroppenstedt RM, Stackebrandt E.** The genus *Nocardiopsis* represents a phylogenetically coherent taxon and a distinct actinomycete lineage: proposal of *Nocardiopsiaceae* fam. nov. *Int J Syst Bacteriol.* 1996: 46: 28–96.
34. **Yoon SH, Ha SM, Kwon S, Lim J, Kim Y, *et al*.** Introducing EzBioCloud: A taxonomically united database of 16S rRNA and whole genome assemblies. *Int J Syst Evol Microbiol*. 2017: 67: 1613-161.
35. **Stamatakis A, Hoover P, Rougemont J.** A rapid bootstrap algorithm for the RAxML web servers. *Syst Biol.* 2008: 57:758-771
36. **Swofford DL.** PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods), Version 4.0 b10. 2000. Sinauer Associates, Sunderland Pattengale
37. **Montero-Calasanz MdC, Göker M, Rohde M, Schumann P, Pötter G, *et al.*** *Geodermatophilus siccatus* sp. nov., isolated from arid sand of the Saharan desert in Chad. *Antonie van Leeuwenhoek* 2013;103:449-456.
38. **Meier-Kolthoff JP, Hahnke RL, Petersen J, Scheuner C, Michael V *et al*.** Complete genome sequence of DSM 30083^{T}, the type strain (U5/41^{T}) of *Escherichia coli*, and a proposal for delineating subspecies in microbial taxonomy. *Stand Genomic Sci* 2014;10:2.

39. **Meier-Kolthoff JP, Auch AF, Klenk H-P, Göker M.** Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics* 2013;14:60.
40. **Hess PN, De Moraes Russo CA.** An empirical test of the midpoint rooting method. *Biol J Linn Soc*. 2007: 92:669-674
41. **Meier-Kolthoff JP, Göker M, Spröer C, Klenk H-P.** When should a DDH experiment be mandatory in microbial taxonomy? *Arch Microbiol* 2013;195**:** 413-418.
42. **Cashion P, Hodler-Franklin MA, McCully J, Franklin M.** A rapid method for base ratio determination of bacterial DNA. *Anal Biochem* 1977;81: 461-466.
43. **De Ley J, Cattoir H, Reynaerts A.** The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* 1970;12(1): 133-142.
44. **Huss VAR, Festl H, Schleifer KH.** Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. *Syst Appl Microbiol* 1983; 4(2): 184-192.
45. **Auch AF, von Jan M, Klenk H-P, Göker M.** Digital DNA: DNA hybridization for microbial species delineation by means of genome-to-genome comparison. *Stand Genom Sci* 2010;2: 117-134.
46. **Normand P, Benson DR.** Family IV. *Geodermatophilaceae* 2006, 2277^{VP} (Effective publication: Normand, Orso, Cournoyer, Jeannin, Chapelon, Dawson, Evtuschenko and Misra, 1996, 8). In Goodfellow M, Kämpfer, P., Busse, H.-J., Trujillo, M.E., Suzuki, K.-I., Ludwig, W., Whitman, W.B. (eds), Bergey's Manual of Systematic Bacteriology, Part A. vol 5, 2nd edn. Springer, New York, 2012 p528.
47. **Hezbri K, Louati M, Nouioui I, Gtari M, Rohde M, *et al.*** *Blastococcus capsensis* sp. nov., isolated from and archaeological Roman pool and emended description of the genus *Blastococcus, B. aggregatus, B. saxobsidens, B. jejuensis and B. endophyticus*. *Int J Syst Evol Microbiol* 2016;66:4864-4872.
48. **Wayne LG, Brenner DJ, Colwell RR, Grimont PaD, Kandler O, *et al.***Report of the Ad Hoc Committee on Reconciliation of Approaches to Bacterial Systematics. *Int J Syst Bacteriol* 1987;37:463-464.
49. **Montero-Calasanz MdC, Göker M, Rohde M, Spröer C, Schumann P, *et al*.** *Chryseobacterium hispalense* sp. nov., a plant growth-promoting bacterium isolated from a rainwater pond in an olive plant nursery, and emended descriptions of *Chryseobacterium defluvii, Chryseobacterium indologenes, Chryseobacterium wanjuense* and *Chryseobacterium gregarium*. *Int J Syst Evol Microbiol.* 2013: 63: 4386-4395.

Table 1. Differential phenotypic characteristics of strain BC 501^T and the type strains of other species of the genus *Modestobacter*.

Strains: 1, *M. italicus* sp. nov. BC 501^T; 2, *M. caceresii* KNN 45-2b^T; 3, '*M. lacusdianchii*' KCTC 39600; 4, *M. lapidis* MON 3.1^T; 5, *M. marinus* DSM 45201^T; 6, *M. multiseptatus* DSM 44406^T; 7, *M. muralis* MDVD1^T; 8, *M. roseus* DSM 45764^T; 9, *M. versicolor* DSM 16678^T. Data are from this study unless indicated otherwise.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Colony morphology: Colour switch/dark colour	P/P	P/P	A/A	P/P	P/P	A/A	P/P	A/A	P/P
According to Biolog GENIII microplates, it oxidises:									
Dextrin	+	+	+	+	+	-	+/-	+	+/-
D-Maltose	-	+	+/-	+	+	+	+	+	+/-
β-Gentiobiose	-	+/-	+	+	+	+	+	-	+
α-D-Lactose	-	-	+	+	+	+	+	+	-
D-Melibiose	-	+/-	+/-	+	+/-	+	+	-	-
β-Methyl-D-Glucoside	-	+	+	+	+	+	+/-	+/-	-
D-Salicin	+	+	+	+/-	+	+	+	+	-
N-Acetyl-D-Glucosamine	-	+	+	+	+	+	+	+	+
N-Acetyl-β-D-Mannosamine	-	+	+/-	+	+	+	+	+/-	+
N-Acetyl-D-Galactosamine	-	-	-	+	+	+	+/-	+/-	-
L-Fucose	-	+	+	+	+/-	+	+	-	+
Sodium Lactate	+	-	-	-	+	-	+	+	+
D-Sorbitol	-	+	+	+	+	+/-	+	+/-	+
D-Glucose-6-Phosphate	-	+	-	+	+	+	+/-	+/-	+
Gelatin	-	+	+/-	-	+	-	+/-	-	-
Glycil-Proline	-	-	-	-	+/-	+	-	-	-
L-Glutamic Acid	-	+	-	+/-	+	+	+/-	+	+/-

L-Pyroglutamic Acid	-	-	-	-	+	+	-	-	+/-
L-Serine	-	-	+	-	+	-	-	+/-	+/-
Pectin	-	+	+	-	+/-	-	+	+	+
D-Galacturonic Acid	-	-	+	+	-	+	-	-	+/-
Glucuronamide	-	+	-	+	-	+	-	-	+/-
p-Hydroxy-Phenylacetic Acid	-	+	-	-	-	-	-	+	-
Methyl Pyruvate	-	+/-	-	-	-	-	-	+	+/-
Citric Acid	-	-	+	-	-	+	-	-	+
D-Malic Acid	-	+	+	+/-	-	+	+	+	-
L-Malic Acid	-	-	+	+	+	+	+	+	+
Potassium Tellurite	+	-	+/-	-	-	-	+	+	+
Tween 40	+	+	+	+	+/-	+	+	+	-
Polar lipids	DPG, PE, PI, GPI	DPG, PG, PE, PI, PIM[¥]	DPG, PE, PI, PIM, PL[Ⓠ]	DPG, PG, PE, PI, PIM[§]	DPG, PG, PE, PI, APL, GL, PL	DPG, PE, PI, GL, 2APL,	DPG, PG, PE, PI, PIM[§]	DPG, PG, PE, PI, GPI, AL, 2APL	DPG, PG, PE, PI, PL, GPI
Diagnostic sugar	Rib, Ara, Man, Glu, Gal	Rham, Rib, Ara, Glu[¥]	Rham, Rib, Ara, Man, Gluc, Gal[Ⓠ]	Rib, Ara, Glu, Gal[§]	Rib, Ara, Man, Glu	Rib, Man, Glu,	Rib, Glu, Gal[§]	Rib, Ara, Man, Glu, Gal	Rham, Rib, Man, Glu, Gal
Menaquinones(MK)[#*]	MK-9(H_4), MK-8(H4), MK-9(H_2)	MK-9(H_4)[¥]	MK-9(H_4)[Ⓠ]	MK-9(H_4)[§]	MK-9(H_4), MK-9, MK-10(H_4)	MK-9(H_4), MK-9(H_6)	MK-9(H_4)[§]	MK-9(H_4), MK-9, MK-8(H_4)	MK-9(H_4), MK-9, MK-9(H_2)

P, present; A, absent; +, positive reaction; –, negative reaction; +/-, ambiguous; DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PI, phosphatidylinositol; GPI, glycophosphoinositol; PIM, phosphoinositolmannoside; AL, aminolipids; APL, aminophospholipids; GL, glycolipid; PL, unidentified phospholipid; Ara, arabinose; Gal, galactose; Glu, glucose; Man, mannose; Rham, rhamnose; Rib, ribose; MK, menaquinones.

[#]The components are listed in decreasing order of quantity.
[*]Only components making up ≥ 1 % peak area ratio are shown
[¥] Data are from Busarakam et al. [3]
[§] Data are from Trujillo et al. [5]
[Ⓠ]Data are from Zhang et al. [4]

Figure legends

Fig. 1. Polar lipids profiling of strain BC 501^T (a), *M. marinus* DSM 45201^T (b), *M. multiseptatus* DSM 44406^T (c), *M. roseus* DSM 45764^T (d) and *M. versicolor* DSM 16678^T (e) after separation by two-dimensional TLC using the solvents chloroform:methanol:water (65:25:4; v:v:v) in the first dimension and chloroform:methanol:aceticacid:water (80:12:15:4; v:v:v:v) in the second one. Plates

were sprayed with molybdatophosphoric acid (3.5 %; Merck^{TM}) for detection of the total polar lipids. DPG, diphosphadidylglycerol; PG, phosphadidylglycerol; PE, phosphatidethanolamine; PC, phosphatidylcholine; PI, phosphatidylinositol; GPI, glycophosphatidylinositol; APL 1-3, unidentified aminophospholipids; GL, unidentified glycolipid; PL, unidentified phospholipid; AL 1-5, aminolipid; L1-8, unidentified lipids. All data are from this study.

Fig. 2. Maximum likelihood phylogenetic treeing based on the 16S rRNA gene sequences and showing BC 501^{T} phylogenetic affiliation within *Geodermatophilaceae*. Only bootstrap values higher than 50 % are shown above the branches.